

### Remarks

#### Oath/Declaration

The Examiner has indicated that the Declaration is defective on the grounds that (i) it does not identify the mailing address of each inventor and (ii) it does not identify the U.S. provisional application to which priority is claimed. Applicants submit that the information missing in the Declaration is not necessary for further consideration of the claims and therefore, in accordance with 37 C.F.R. 111(b), Applicants hereby requests that the requirement to correct the defects be held in abeyance until an indication of allowable subject matter is received.

#### Amendment to the Specification

The title has been amended to more clearly reflect the scope of the claimed invention. As indicated throughout the specification, the claimed invention has a wide variety of uses in addition to sample preparation for 2-DGE.

#### Rejections under 35 U.S.C. § 112

Claim 27 stands rejected as being indefinite on the ground that the preamble does not correspond to the method outcome in that the preamble recites a method for separating ligands from a sample while the final method step recites analyzing the remaining ligands. Claim 27 has been amended to recite indicate that the method includes recovery of a sample and to remove the final step so that the preamble and methods steps correspond. During the telephonic interview held Jan. 26, 2005, the Examiners indicated that the original claim was confusing in that it used the term "ligand" to refer to the specific predefined substances in the sample that are removed by the receptors and also to refer to substances that remain in the sample for subsequent analysis after removal of the specific predefined substances. The claim has therefore been amended to use the alternate term "components" to refer to substances remaining after the removal of the predefined ligands. The specification uses both the terms "ligand" and "component" to refer to substances that may be present in a sample, and the change is made simply for purposes of clarification. The claim language has also been simplified relative to the draft claim in order to enhance clarity, as suggested by the Examiners. Support for the addition of the "recovering" step is found throughout the specification, e.g., at p. 28, ~lines 3-4, indicating that samples

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generated by the subtraction process were collected. During the interview the Examiners indicated that these amendments would be of considerable value in addressing their concerns.

Claims 35-38 stand rejected as being indefinite on the ground that the phrase "the process" lacks antecedent basis. The claims have accordingly been amended to replace "the process" with "the method" to provide antecedent basis. It is evident from the original wording of the claims that "the process" is intended to refer to the method recited in the claim on which the amended claim depends. Claim 44 has also been amended in the same manner. Withdrawal of the rejection is respectfully requested.

Claims 35 and 37 stand rejected as being indefinite on the grounds that the recitations of "the receptors" and "the same receptors" lack antecedent bases. Claims 35 and 36 have been amended so that the amended claims are dependent on claim 34, which contains the phrase "the receptors", thereby providing proper antecedent basis. Applicants respectfully submit that it is now clear that phrase "the same receptors" in claim 37 refers to "the receptors" referred to in amended claim 35. Withdrawal of the rejection is respectfully requested.

Claims 41-42 stand rejected as being indefinite on the ground that the recitation of "division" is indefinite because it is not clear whether "division" is based on a numerical division and/or a functional and/or structural division. While Applicant maintain that the meaning of "division" is clear from the specification, the term has been removed from the claims. In claim 42, to indicate that the receptors are distinct, the claim has been amended to recite that the receptors have different binding specificities, as indicated on p. 15, lines ~29-31 through p. 16, line 3. Withdrawal of the rejection is respectfully requested.

Claim 44 stands rejected as being indefinite on the ground that the recitation of "modified ligand-containing sample" lacks antecedent basis. Applicants have amended claim 27 to refer simply to a "modified sample" to provide antecedent basis and have amended claim 44 accordingly, but submit that the amendment does not change the meaning or scope of either claim 27 or claim 44. Withdrawal of the rejection is respectfully requested.

#### Rejections under 35 U.S.C. § 102

Claims 27-42 and 44 stand rejected as being anticipated by Wheatley, 603 J. Chromatogr. 273 (1992), hereinafter "Wheatley". The Office Action states that Wheatley teaches a method for separating ligands comprising removing at least two specific predefined ligands and

analyzing the remaining ligands. As discussed during the Interview, Applicants have amended claim 27 to recite a particular embodiment of the invention in which a plurality of components remains in the sample following removal of the at least two specific predefined ligands, resulting in a modified sample comprising a plurality of components to be analyzed. Applicants submit that Wheatley neither teaches nor suggests such a method for each of the following reasons:

As discussed in the Interview, Wheatley does not teach a method for separating ligands from a sample *for analysis of remaining components*. Instead, Wheatley teaches a method for *purifying a known protein* of interest from a sample by removing protein "contaminants" in the sample using affinity chromatography. Wheatley's focus is entirely on purification of the known protein of interest rather than on facilitating analysis of the modified sample that remains after removal of the specific "contaminants". Thus Wheatley does not teach a method of sample preparation involving removal of specific ligands for analysis of remaining components. The protein present in Wheatley's sample together with the contaminating proteins is not a component to be analyzed but rather a protein to be purified.

As also discussed during the Interview, claim 27 as amended recites that a *plurality* of components remains in the sample following removal of the at least two specific predefined ligands. In contrast, Wheatley describes an experiment in which a mixture containing three proteins – albumin, transferrin, and IgG – was injected onto an anti-(albumin, transferrin) affinity column, and two of the proteins – albumin and transferrin – were removed from the mixture (see legend of Fig. 3(b) and left column of text on p. 276). Following removal of albumin and transferrin, the modified sample contained only a single protein rather than a plurality.

In summary, Wheatley does not teach a method for separating ligands from a sample for analysis of remaining components as taught in amended claim 27 because (i) Wheatley's method is directed to purification of a ligand and thus is not a method of sample preparation for analysis of remaining components; (ii) the protein present in Wheatley's sample together with the contaminating ligands is not a protein to be analyzed but is instead a protein to be purified; and (iii) Wheatley does not recover a modified sample comprising a plurality of components to be analyzed. Withdrawal of the rejection of claim 27 and claims dependent therefrom is respectfully requested.

Applicants note that the Examiner acknowledged in the Office Action that Wheatley does not teach a method in which three or four ligands are removed, as recited in claims 28 and 29. Withdrawal of the rejection of these claims is respectfully requested.

With respect to claims that were not specifically discussed during the Interview, Applicants submit the following remarks. Regarding claim 34, the Office Action states that Wheatley describes a method for separating ligands wherein the bound ligands are removed from the receptor and refers to Wheatley's statement that "the proteins were eluted using a linear salt gradient". Since amended claim 27 is novel, claim 34 (which depends on claim 34) is also novel regardless of whether Wheatley teaches removal of bound ligands from the receptors. Furthermore, upon examining Fig. 2(b) and 3(b) it is evident that Wheatley does not teach removal of bound ligands from the receptors since the linear salt gradient did not in fact remove the ligands (albumin and transferrin) bound to the receptors. Had it done so, these proteins would have been evident in the material that flowed through the column. Wheatley states that, "The integrity of the antigen-antibody complexes formed between albumin, transferrin and their antibodies in the affinity pre-column was apparently unperturbed by the application of the salt gradient, as indicated by the absence of these proteins in the chromatograms shown in Figs. 2b and 3b." (p. 277, upper left column). Therefore Wheatley does not teach a method in which the bound ligands are removed from the receptors. Withdrawal of the rejection of claim 34 is respectfully requested.

The Office Action states with respect to claim 35 that Wheatley teaches a method for separating ligands wherein the receptors are reused and refers to p. 275, col. 2, which states that "The chromatographic conditions were identical for all injections and are described in Fig. 2." This statement does not teach or suggest that the receptors were or could be reused but simply indicates that in the two experiments presented in Fig. 2, identical injection conditions were used. Fig. 2 compares results obtained when a mixture of albumin and transferrin was injected onto either the SCX column (Fig. 2(a)) or an albumin-transferrin affinity column preceding the SCX column (Fig. 2(b)). Thus it is evident that the affinity column, which contains the receptors, was used only once in the experiment of Fig. 2. Although other experiments involving use of affinity columns are described in Wheatley, Wheatley does not teach that the same affinity column was reused rather than, for example, using different affinity columns with the same type

of antibodies attached for the different experiments. Furthermore, even if the same affinity column was reused, this does not indicate that the *receptors themselves* were reused in the sense of binding additional ligand. There is no teaching or suggestion in Wheatley regarding removal of a bound ligand from a receptor. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103(a)

Claims 28-29 and 40-42 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Wheatley in view of U.S. Patent No. 5,372,783, to Lackie, hereinafter "Lackie". The Office Action states that while Wheatley does not teach a method wherein at least three or four ligands are removed, Lackie teaches a chromatography column in which at least three or four ligands are removed. The Office Action further states that Lackie teaches a chromatography column wherein at least one receptor (or receptor division) is selectively removable from another receptor and that Lackie teaches a chromatography column in which at least two divisions of receptor are immobilized in two different predefined locations.

Applicants respectfully submit that the combination of Wheatley and Lackie does not render claims 28-29 or 40-42 obvious as read with original claim 27. However, since claim 27 has been amended, the rejection will be addressed in reference to the claims as read in light of the amendment of claim 27. As discussed during the Interview, Wheatley teaches removing ligands from a sample for purposes of purifying a known protein of interest. Lackie teaches detection of one or more specific predefined ligands in a sample by flowing the sample through a flow cell that may contain immobilized receptors that bind to the ligands to be detected. The Office Action states that it would have been obvious to have "provided a method for separating ligands, as taught by Wheatley, with the modified chromatography column of Lackie because Lackie teaches that his chromatography column has the ability to incorporate several different receptors in order to simultaneously assay several different ligands." The Office Action further states that "Wheatley suggests that his method for separating ligands can be extended beyond a simple two protein extraction to also assay multi-component contaminant mixtures." However, Applicants submit that Lackie does not teach a chromatography column but rather a flow cell. A chromatography column would generally be understood in the art to be a device that could be used for *separation* of components in a sample. Lackie does not teach that his device is useful for separation of components in a sample but rather for *detection* of specified components in a

sample. Wheatley does not teach that his method is useful for *assaying* contaminant mixtures but rather for *purifying* a specified component of a sample by removing minor contaminants. Since Wheatley and Lackie teach that their devices and methods are useful for distinct and nonoverlapping purposes, there would be no motivation to combine the two references.

Even if motivation to combine existed, the combination of Wheatley and Lackie does not teach each of the features of the claimed invention. In particular, neither Wheatley nor Lackie teaches or suggests a method for separating ligands from a sample for analysis of components remaining in the sample. Wheatley provides a sample containing two predefined proteins and a known protein to be purified while Lackie teaches *detection* of predefined ligands. Furthermore, neither Wheatley nor Lackie teaches or suggests that a plurality of components is present in the sample after application of their respective methods, as recited in amended claim 27. Therefore, the combination of Wheatley and Lackie does not teach each of the features of the claimed invention.

In summary, as discussed during the Interview, since there is no motivation to combine the teachings of Wheatley and Lackie and since the combination does not teach each of the features of the invention, Applicants submit that claims 28-29 and 40-42 are not obvious. Withdrawal of the rejection is respectfully requested.

#### Double Patenting

Claims 27-42 and 44 stand provisionally rejected as claiming the same invention as that of claims 27-42 and 44 of copending Application No. 10/250,898. Applicants respectfully request deferral of this provisional rejection until such time, if any, that it matures into an actual rejection. Applicants further note that claims 27-42 and 44 of the copending application have been withdrawn from consideration because of a Restriction Requirement.

#### New Claims and Claim Amendments

As mentioned above, claim 27 has been amended to remove the "analyzing" step. Claim 39 has been amended to include an analysis step. New claim 45 and various other claims have been added to encompass a method for preparing and analyzing a sample. Support for these claims is found in original claim 27 and throughout the specification. New claim 46 recites that the analyzing step comprises identifying at least one physical characteristic of at least one

component remaining in the modified sample, and new claim 47 recites particular physical characteristics that may be determined. Support for these claims is found throughout the specification, which teaches the use of various methods such as electrophoresis, mass spectrometry, etc., which are known in the art to identify various physical characteristics of analytes, including the physical characteristics listed in claim 47. New claim 48 recites that the analyzing step comprises performing mass spectrometry, 2D gel electrophoresis, chromatography, an immunoassay, a binding assay, or any combination of the foregoing. Support for this claim is found at p. 3, lines 1-4 and at p. 5, lines ~15-16, etc. New claim 49 recites that the analyzing step comprises identifying the component, which is supported at numerous locations throughout the specification, e.g., at p.4, lines ~24-25, indicating that identification techniques can be applied to the sample following removal of the ligands. New claim 51 recites that the analyzing step comprises determining the presence, abundance, or both, of the component, and new claim 52 recites that the presence, abundance, or both of the component in the sample was previously unknown. Support for these claims is found at p. 6, lines 15-18 (referring to detection and quantitation) and in Preparative Example B, p. 25, lines 7-11, indicating that following removal of abundant proteins, additional proteins that were previously undetectable (such that their presence, abundance, or both was unknown) became visible, and in Examples 7-9 (pp. 32-33), indicating that many low abundance proteins became visible in the immunodepleted sample. New claims 52-58 are similar to claims 45-51 except that they apply to a plurality of components. Support for these claims is found as described for claims 52-58.

New claim 59 recites that the specific predefined receptors were selected to facilitate analysis of components remaining in the modified sample. Support for this claim is found throughout the specification, e.g., at p. 3, lines 1-11, at p.7, lines 7-8, reciting that the proteins to be removed from the sample are removed by exposing the sample to receptors that specifically bind to the proteins to be removed, and at p. 7, lines 20-23, indicating that any ligand (not just proteins) can be removed.

New claim 60 recites that the method may further comprise concentrating components remaining in the modified sample, adding a buffer to the modified sample, dialyzing the modified sample, lyophilizing the modified sample, or any combination of the foregoing. Support is found at p. 18, line 30 – p. 19, line 2.

New claim 61 recites that the modified sample has improved characteristics for analysis of ligands remaining in the sample. Support is found at p. 3, lines 1-11 and elsewhere in the specification.

New claim 62 recites that at least one of the specific predefined ligands is present at higher abundance than at least one of the plurality of components remaining in the sample. Support for claim 64 is found at p. 4, lines ~26-28, stating that the invention may be used to remove abundant proteins from a sample to enable resolution of less abundant proteins, thereby indicating that the sample contains at least one predefined ligand that is present at higher abundance than one or more less abundant proteins. Further support is found throughout the specification including in Example 1 (pp. 28-29) describing removal of 6 abundant proteins from a serum sample followed by analysis of less abundant proteins remaining in the sample.

New claims 63-69 recite that the removing step comprises contacting the sample with one of a variety of inventive affinity binding compositions. New claim 70 specifies that the contacting step comprises passing the sample through a column containing an inventive affinity binding composition. Support for these claims is found throughout the specification, e.g., in Example 1 (pp. 28-29) and in original claims 1-12.

New claim 77 recites that the removing step comprises contacting the sample with an affinity binding composition comprising: a plurality of solid phase matrices arranged such that each solid phase matrix is in contact with at least one other solid phase matrix; and a plurality of receptors, the plurality of receptors comprising receptors of a plurality of receptor types, wherein the receptors are immobilized on the plurality of solid phase matrices such that each receptor type is immobilized on a single matrix and each receptor type binds specifically to a different ligand. Support for this claim is found e.g., at p. 11, line 30 and in Example 1, p. 28.

New claims 71, 78, 86, and 93 recite that each solid phase matrix in the affinity binding compositions recited in claims 70, 77, 84, and 91, respectively, comprises a plurality of particles. Support is found throughout the specification, e.g., at p. 10, lines 10-12, describing POROS particles as suitable solid phase matrices and in the Examples, which describe use of POROS particles as solid phase matrices.

New claims 72, 79, and 87 recite that the solid phase matrices, each comprising a plurality of particles, are present as a mixture. Support is found, e.g., in original claims 19 and 24.

New claim 73 recites that the first receptor is not immobilized on the second solid phase matrix. Support is found at p. 17, lines 25-26.

New claims 74, 80, 88, and 94 recite that the receptors are antibodies, antibody fragments, or lectins. Support is found in original claim 6, at p. 7, line 10, and at p. 8, lines 21-23.

New claims 75, 81, 89, and 95 recite that the receptors are recombinantly produced. Support is found at p. 7, line 31 and at p. 8, line 14.

New claims 76, 82, 90, and 96 recite that at least one receptor is selected from the group consisting of metals, cofactors, nucleic acids, aptamers, combinatorial compounds, combinatorial peptides, combinatorial oligomers and combinatorial polymers. Support is found at p. 7, lines 11-16.

New claim 84 recites that the removing step comprises contacting the sample with an affinity binding composition comprising: a plurality of solid phase matrices arranged such that each solid phase matrix is in contact with at least one other solid phase matrix; and a plurality of receptors having different ligand binding specificities, wherein the receptors are immobilized on the plurality of solid phase matrices such that each solid phase matrix has a different ligand binding specificity. Support for this claim is found, e.g., at p. 17, line 25-28.

New claims 83 and 85 specify that the contacting step comprises passing the sample through a column containing the inventive affinity binding composition of claim 77 or 84, respectively. Support is found, e.g., in original claims 8-12 and in Example 1 (pp. 28-29).

New claim 91 recites that the removing step comprises passing the sample through first and second solid phase matrices, the first and second solid phase matrices having different ligand binding specificities and being arranged in layers. New claim 97 recites that the solid phase matrices may be separated by a permeable membrane. Support for these claims is found at p. 15, line 29 – p. 16, line 1, and at p. 16, lines 27-28. New claim 92 recites that the solid phase matrices are in a column. Support is found, e.g., in original claims 8-12.

New claim 98 recites a method for separating ligands from a sample and analyzing remaining ligands comprising: providing a sample comprising at least two specific predefined ligands and one or more additional ligands; removing at least two of the specific predefined ligands from the sample; and analyzing at least 100 ligands remaining in the sample. Support is

found at p. 3, lines 16-19, indicating that using the present invention hundreds (i.e., at least one hundred) of additional proteins can be analyzed.

New claim 99 recites particular analysis methods. Support for this claim is found as for claim 48.

New claim 100 recites a method for separating ligands from a sample for analysis of remaining components comprising: providing a sample comprising at least two specific predefined ligands and a plurality of additional ligands to be analyzed; and removing a plurality of the specific predefined ligands from the sample, thereby producing a modified sample, wherein removal of the plurality of specific predefined ligands renders at least one remaining component detectable by a detection method that was unable to detect the other ligand prior to removal of the plurality of specific predefined ligands. The claim is supported by original claim 27 and throughout the specification, e.g., in Preparative Example B, p. 25, lines 7-11, indicating that following removal of abundant proteins, additional proteins that were previously undetectable became visible. Additional support is found at Example 5, p. 30, lines ~18-20. New claim 101 recites that the method of claim 100 allows quantitation of at least 50% more components in the modified sample than could be quantitated in the sample containing the plurality of specific predefined ligands. Support is found at p. 22, line 21. New claim 102 recites that the method of claim 100 further comprises the step of analyzing at least one component remaining in the modified sample. Support for this claims is found in original claim 1

New claim 103 recites the method of claim 32 wherein at least 90% by weight of all proteins in the sample are removed. Support for this claim is found at p. 20, line 3.

New claim 104 recites that at least one of the predefined ligands in the method of claim 27 is selected from the group consisting of one or more immunoglobulins, albumin, transferrin, haptoglobin,  $\alpha_1$ -antitrypsin, hemopexin,  $\alpha_1$ -acid glycoprotein, myosin, transthyretin,  $\alpha_1$ -antichymotrypsin, apolipoprotein A1,  $\alpha_2$ -macroglobulin, fibrinogen, and prealbumin. New claim 105 recites that at least two of the predefined ligands are selected from the foregoing list. New claim 106 recites that at least three of the predefined ligands are selected from the foregoing list. New claim 107 recites that at least four of the predefined ligands in the method of claim 27 are selected from the list. Support for these claims is found at p. 19, lines 15-18 and p. 25, Table 1.


New claim 108 recites that the method of claim 27 further comprises a step of subjecting the sample to deglycosylation. New claim 109 recites that the method of claim 27 further comprises a step of subjecting the modified sample to deglycosylation. Support for these claims is found at p. 19, lines 3-7.

In conclusion, in view of the amendments and remarks presented herein, none of the cited art anticipates any of the claims pending in the instant application nor renders them obvious, and the application complies with the requirements of 35 U.S.C. §112. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

Please charge any fees associated with this filing, including the fee for a 2 month extension of time and any additional claims fees or other fees that are due, or apply any credits, to Deposit Account No. 50-1078.

Respectfully submitted,

  
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